IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of :

Kouichirou HIRATA et al. : **Attn: BOX PCT**

Serial No. NEW : **Docket No. 2001_1888A**

Filed December 26, 2001 :

METHOD FOR THE DETECTION OF *STREPTOCOCCUS SOBRINUS* AND ANTIBODY USED THEREFOR
[Corresponding to PCT/JP01/03502
Filed April 24, 2001]

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents,
Washington, DC 20231

Sir:

Prior to calculating the filing fee, please amend the above-identified application as follows:

IN THE SPECIFICATION

Page 1, immediately after the title, please insert:

This application is a 371 of PCT/JP01/03502 filed April 24, 2001.

IN THE CLAIMS

Please amend the claims as follows:

5. **(Amended)** A method as claimed in claim 1 wherein the immune complex is assayed by an immunochromatographic technique.

ATTACHMENT E

10. **(Amended)** A diagnostic method as claimed in claim 6 wherein step (c) is carried out in the coexistence of the antibody (S antibody) with an antibody binding specifically with *Streptococcus mutans* (M antibody), or in addition to step (c), another step similar to step (c) is carried out by using M antibody in place of S antibody; the resulting immune complex derived from M antibody is also assayed; and the amount of this complex is also evaluated as an index to a risk of dental caries.

12. **(Amended)** A diagnostic method as claimed in claim 6 wherein the one or more immune complexes are assayed by an immunochromatographic technique.

13. **(Amended)** A diagnostic method as claimed in claim 6 wherein the test fluid is untreated saliva.

REMARKS

The specification has been amended to reflect the 371 status.

In addition, the multiple dependencies of the claims have been removed, so as to reduce the PTO filing fee and to eliminate improper multiple dependencies.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached pages are captioned "**Version with markings to show changes made**".

Favorable action on the merits is solicited.

Respectfully submitted,

Kouichirou HIRATA et al.

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DESCRIPTION

METHOD FOR THE DETECTION OF *STREPTOCOCCUS SOBRINUS* AND ANTIBODY USED THEREFOR

This application is a 371 of PCT/JP01/03502 filed April 24, 2001.

Technical Field

This invention relates to an immunological method for the assay of *Streptococcus sobrinus* (*Streptococcus* may hereinafter be abbreviated as *S.*), a method for diagnosing the degree of risk of dental caries on the basis of the assay, and an antibody and means for use in these methods.

Background Art

It is generally known that a class of lactic acid fermentative bacteria called mutans streptococci are closely associated with the onset of dental caries. Accordingly, the degree of risk of dental caries is being judged by examining the quantity of mutans streptococci present in the oral cavity of human subjects, and assay kits therefor are commercially available. It is said that there is a risk of dental caries when the concentration of mutans streptococci in saliva is in the range of 10^5 to 10^6 cells/ml and there is a high risk when it is greater than 10^6 cells/ml.

These mutans streptococci are classified into seven types which are serologically and genetically different from each other. Specifically, they are *Streptococcus cricetus* (serotype a), *Streptococcus rattus* (serotype b), *Streptococcus mutans* (serotypes c, e and f), *Streptococcus ferus* (serotype c), *Streptococcus macacae* (serotype c), *Streptococcus sobrinus* (serotypes d and g), and *Streptococcus downey* (serotype h).

Now, it has been proved that, among these *mutans* streptococci, only two bacteria, namely *Streptococcus mutans* and

CLAIMS

1. A method for the detection of *Streptococcus sobrinus* in a test fluid suspected of containing *Streptococcus sobrinus* and *Streptococcus mutans*, said method comprising the steps of

(A) providing an antibody whose binding ability for *Streptococcus sobrinus* is not less than 100 times that for *Streptococcus mutans*;

(B) bringing the antibody into contact with the test fluid to form an immune complex; and

(C) assaying the immune complex.

2. A method as claimed in claim 1 wherein the antibody whose binding ability for *Streptococcus sobrinus* is not less than 100 times that for *Streptococcus mutans* is a polyclonal antibody.

3. A method as claimed in claim 2 wherein the binding ability for *Streptococcus sobrinus* is determined with respect to the serotype d and g strains of the bacterial species, and the mutual ratio between the binding abilities for the serotype d and g strains is within 2.

4. A method as claimed in claim 1 wherein the test fluid suspected of containing *Streptococcus sobrinus* and *Streptococcus mutans* contains *Streptococcus sobrinus* at a concentration of 10^5 to 10^7 cells/ml.

(amended)

5. A method as claimed in ~~any one of~~ claims 1 ~~to 4~~ wherein the immune complex is assayed by an immunochromatographic technique.

6. A diagnostic method for judging the degree of risk of dental caries in a human subject, said method comprising the steps of

(a) preparing a test fluid derived from the saliva and/or

dental plaque of the subject;

(b) providing an antibody whose binding ability for *Streptococcus sobrinus* is not less than 100 times that for *Streptococcus mutans*;

(c) bringing the test fluid prepared in step (a) into contact with the antibody provided in step (b) to form an immune complex; and

(d) assaying the immune complex, and evaluating its amount as an index to a risk of dental caries.

7. A diagnostic method as claimed in claim 6 wherein the antibody whose binding ability for *Streptococcus sobrinus* is not less than 100 times that for *Streptococcus mutans* is a polyclonal antibody.

8. A diagnostic method as claimed in claim 7 wherein the binding ability for *Streptococcus sobrinus* is determined with respect to the serotype d and g strains of the bacterial species, and the mutual ratio between the binding abilities for the serotype d and g strains is within 2.

9. A diagnostic method as claimed in claim 6 wherein the test fluid contains *Streptococcus sobrinus* at a concentration of 10^5 to 10^7 cells/ml.

10. (Amended) A diagnostic method as claimed in ~~any one of~~ claims 6 ~~to 9~~ wherein step (c) is carried out in the coexistence of the antibody (S antibody) with an antibody binding specifically with *Streptococcus mutans* (M antibody), or in addition to step (c), another step similar to step (c) is carried out by using M antibody in place of S antibody; the resulting immune complex derived from M antibody is also assayed; and the amount of this complex is also evaluated as an index

to a risk of dental caries.

11. A diagnostic method as claimed in claim 10 wherein an antibody binding specifically with *Streptococcus mutans* and *Streptococcus sobrinus* (MS antibody) is used in place of M antibody.

12. (Amended) A diagnostic method as claimed in ~~any one of~~ claims 6 ~~to 11~~ wherein the one or more immune complexes are assayed by an immunochromatographic technique.

13. (Amended) A diagnostic method as claimed in ~~any one of~~ claims 6 ~~to 12~~ wherein the test fluid is untreated saliva.

14. An immunoassay kit or a diagnostic kit for judging the degree of risk of dental caries in human subjects, said kit including an antibody whose binding ability for *Streptococcus sobrinus* is not less than 100 times that for *Streptococcus mutans*; and if necessary, an antibody binding specifically with *Streptococcus mutans*, or an antibody binding specifically with *Streptococcus mutans* and *Streptococcus sobrinus*.

15. An immunochromatographic strip comprising a sample pad for absorbing and holding a test fluid temporarily, a conjugate pad for holding a labeled antibody temporarily, and a development membrane having a detection antibody immobilized thereto and allowing the development of the test fluid absorbed and held temporarily in the sample pad and the labeled antibody flowing out of the conjugate pad together with the test fluid, wherein the sample pad, the conjugate pad and the development membrane are joined together in the order mentioned, said immunochromatographic strip being characterized in that an antibody whose binding ability for *Streptococcus sobrinus* is not less than 100 times that for *Streptococcus mutans* is used as the